The role of the gut microbiome in chronic liver disease: the clinical evidence revised

Katherine JP Schwenger,[1,2] Nayima Clermont-Dejean,[1] Johane P. Allard[1,2,3,*]

Summary

Recent research has suggested a role for the intestinal microbiota in the pathogenesis and potential treatment of a wide range of liver diseases. The intestinal microbiota and bacterial products may contribute to the development of liver diseases through multiple mechanisms including increased intestinal permeability, chronic systemic inflammation, production of short-chain fatty acids and changes in metabolism. This suggests a potential role for pre-, pro- and synbiotic products in the prevention or treatment of some liver diseases. In addition, there is emerging evidence on the effects of faecal microbial transplant. Herein, we discuss the relationship between the intestinal microbiota and liver diseases, as well as reviewing intestinal microbiota-based treatment options that are currently being investigated.

Keywords: microbiome; microbiota; intestinal microbiota; liver diseases; prebiotics; probiotics; fecal transplantation; synbiotics; gut-liver axis.

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Introduction

The human intestinal microbiota (IM) is made up of bacteria, archaea and eukaryotic microorganisms and viruses.[1,2] Currently, there are 1,000 known species of bacteria[3] and approximately 10^{14} microorganisms.[4] Two dominant phyla, Bacteroidetes and Firmicutes, comprise 90% of bacteria in the human digestive tract.[5–7] The IM plays an essential role in the digestion of food, synthesis of vitamins, metabolism, immune system function, inflammation and cell proliferation.[4,8,9] Recently, disturbances in the IM, or dysbiosis, have been associated with several diseases, including a wide range of hepatic disorders.[4,9–12]

Emerging evidence supports the bidirectional relationship between the IM and the liver, which results from the liver receiving 75% of its blood supply from the intestines via the portal vein[13] and the liver releasing bile acids into the biliary tract.[14] As a result, the IM may contribute to liver diseases through several mechanisms that can be influenced by bacterial composition, IM metabolism of bile acids, diet, environmental factors and genetics, with bacteria, bacterial products and metabolites translocating through the intestinal barrier into the portal system, and then the liver.[11,12]

The aim of this review is to outline how the IM and liver interact with each other. We will focus on the IM's role in the pathogenesis and treatment of liver diseases, specifically non-alcoholic fatty liver disease (NAFLD), alcohol-related liver disease (ALD), primary sclerosing cholangitis, primary biliary cholangitis, hepatocellular carcinoma (HCC) and cirrhosis. This review will focus on clinical data and interventions for each of these pathologies.

Intestinal microbiota and liver disease: Overall mechanisms

Bile acid metabolism

Synthesised from cholesterol in the liver, bile acids (BAs) are essential in cholesterol metabolism and lipid digestion.[15] BAs are stored in the gallbladder and are secreted during digestion into the small intestine.[16] Over 95% of BAs are reabsorbed in the terminal ileum and transported back to the liver via the portal vein. BAs promote the absorption of dietary fats, cholesterol and fat-soluble vitamins.[16] In addition, BAs also function as signalling molecules that influence physiological processes,[16] which include the regulation of glucose and lipid metabolism through farnesoid X receptor (FXR) activation and binding of G-protein-coupled bile acid receptor 1.[17–19] BAs can also influence the IM as it has been shown to be directly associated with intestinal mucosal integrity and synthesis of antibacterial peptides.[20] When BAs bind to FXR, antimicrobial peptides, such as angiogenin 1, are produced. These peptides can inhibit IM overgrowth by increasing the intestinal epithelial cell potential to prevent bacterial uptake, improving gut-barrier function.[20] In turn, the IM can influence the size and composition of the BA pool through the conversion of primary to secondary BAs.[21,22] This may subsequently change the composition of the circulating BAs, which act as signalling molecules affecting, for example, lipid and glucose

[1]*Toronto General Hospital, University Health Network, Toronto, Canada;*
[2]*Department of Medicine, University of Toronto, Toronto, Canada;*
[3]*Department of Nutritional Sciences, University of Toronto, Toronto, Canada*

*Corresponding author.
Address: Toronto General Hospital, 585 University Avenue, 9-973, Toronto, ON M5G 2C4; Tel.: 416-340-5159, fax: 416-348-0065.
E-mail address: Dr.Johane.Allard@uhn.ca (J. Allard).

metabolism and predisposing individuals to non-alcoholic fatty liver disease (NAFLD). Therefore, both the dysbiosis of IM and/or imbalance of BAs can contribute to the pathogenesis and progression of liver diseases, which will be discussed.[21,22]

Key points

The intestinal microbiota and bacterial products can directly and indirectly affect the liver through various mechanisms, leading to a wide variety of liver diseases.

This suggests a potential role for pre-, pro- and synbiotic products in the prevention or treatment of some liver diseases.

There is also emerging evidence that faecal microbiota transplant may be an effective treatment for certain liver diseases.

Intestinal permeability

The intestinal epithelium plays an essential role in restricting toxins, antigens and enteric flora from entering the circulation, while selectively permitting the absorption of nutrients across the tight junctions.[23] The intestinal barrier is comprised of enterocytes that are bound to each other by transmembrane proteins including desmosomes, adherens junctions and tight junctions.[23] The intestinal barrier is also strengthened by immunoglobulins, mucins and commensal bacteria.[23] The IM can alter the intestinal barrier by altering tight junctions, degrading the mucus layer or inhibiting the production of mucus, which subsequently increases the permeability of the epithelium.[23] One way in which the IM is associated with increased tight junction permeability is through the presence of luminal endotoxins.[23] Endotoxins found on the outer membrane of gram-negative bacteria increase tight junction permeability by increasing toll-like receptor (TLR)4 expression.[24] Widening of the tight junctions leads to increased intestinal permeability, resulting in increased translocation of bacterial fragments and endotoxins into the portal circulation and subsequently the liver.[25] This in turn can cause systemic and hepatic inflammation and hepatic injury.[25] Bacterial fragments and products can also recruit and activate hepatic immune cells, further contributing to liver disease progression.[25]

Chronic inflammation

The IM contributes to chronic inflammation not only through the production of endotoxins but also through cytokines and inflammasome dysfunction. Translocation of IM-derived endotoxins into the circulatory system increases TLR4 expression, which activates the proinflammatory cytokines tumour necrosis factor-alpha (TNF-a) and interleukin (IL)-6,[26] thus triggering systemic inflammation. Inflammasomes, which consist of leucine-rich-repeat containing proteins and nucleotide-binding domains, govern the cleavage of proinflammatory cytokines. Dysbiosis has been shown to be associated with inflammasome deficiency, specifically NLRP 3 and 6, resulting in the increased expression of TNF-a.[27] The increased activation and production of TLR4 and proinflammatory cytokines from dysbiosis can also lead to the recruitment and activation of hepatic immune cells, contributing to liver disease progression.[27]

Immune system activation

The recruitment and activation of hepatic immune cells can be caused either by local signals or signals from sources such as the IM.[25] The immune system is divided into the innate and adaptive immune systems. The innate immune system defends against microorganisms and toxins, whereas the adaptive immune system is antigen specific and requires self-non-self-recognition.[28] Kupffer cells (KCs) are critical components of the innate immune system, residing within the sinusoidal vascular space.[29] KCs can be activated by various endogenous and exogenous stimuli including endotoxins.[29] Activation of KCs triggers the production of inflammatory cytokines, such as TNF-α, as well as reactive oxygen species (ROS)[29] which can produce tissue damage. These cytokines can also play a key role in regulating the phenotype and function of neighbouring parenchymal and non-parenchymal cells.[29] For instance, cytokines have been shown to polarise and activate the proinflammatory M1 phenotype in KCs.[30]

Natural killer (NK) and natural killer T (NKT) cells may also play a role in the pathogenesis of liver diseases and can be affected by the IM. Recent murine studies have shown that IM-derived antigens could influence the composition and activation of hepatic NKT cells.[31,32] NK cells in the liver play a role in linking the innate and adaptive immune response.[33] Activated NK cells were found to have anti-fibrotic effects, by releasing interferon-γ (IFN-γ) which induces hepatic stellate cell (HSC) cycle arrest and apoptosis.[34] However, IFN-γ also results in hepatocyte apoptosis and thus causes hepatic injury.[34] NKT cells, which can be expressed by hepatocytes and antigen presenting cells, share properties of both T cells and NK cells.[35] NKT cells can secrete cytokines and therefore play a critical role in directing the immune system.[35] They are able to do this through their ability to produce T helper 1 cells, which are proinflammatory, and T helper 2 cells, which are anti-inflammatory.[35] Overall, the activation of hepatic immune cells by the IM could contribute to the pathogenesis of several liver diseases.

Short-chain fatty acids

Another mechanism by which the IM can contribute to liver disease is through the production of short-chain fatty acids (SCFAs). The IM breaks down non-digestible carbohydrates releasing SCFAs in the human gut.[36] The primary SCFAs are acetate, propionate and butyrate, which are metabolised by the muscle, liver and epithelium, respectively.[36] Research has predominately focussed on butyrate, a primary source of energy for colonocytes, which improves colonic barrier function[36] and therefore

positively impacts on intestinal permeability. Butyrate has been shown to improve the gut barrier by induction of tight junction proteins and mucins, specifically Mucin 2[37–39] and enhanced expression of claudin-1.[40] In the liver, butyrate can induce apoptosis and can inhibit cell proliferation in hepatic cells by suppressing sirtuin1 expression while upregulating miR-22 expression.[41] Therefore, butyrate can also inhibit hepatic cancer cells. Butyrate has also been shown to increase satiety, decrease food intake and delay gastric emptying through activation of free fatty acid receptor 3.[42] Free fatty acid receptor 3 upregulates the production of gut hormones peptide YY and glucagon-like peptide-1.[42] Therefore, the IM can affect the metabolism, including diet-induced obesity. Finally, butyrate can also impact on inflammation. In the intestinal tract, studies have found that butyrate binds and activates peroxisome proliferator-activated receptor gamma (PPAR-y), which antagonises nuclear factor-kappa B (NF-kB) transduction, thus causing an anti-inflammatory effect.[43] Therefore, the presence and/or abundance of butyrate produced by the IM could impact on the pathogenesis of liver diseases through several mechanisms.

Choline

Choline is an essential nutrient and a phospholipid component of the cell membrane, which can be metabolised by the IM. There are several mechanisms through which choline deficiency may impact the liver, including[44] decreased very-low density lipoprotein (VLDL) formation, mitochondrial dysfunction and endoplasmic reticulum stress.[44,45] Phosphatidylcholine, which is a phospholipid that contains choline in the headgroup, is a key component of the VLDL envelope. Choline deficiency, either due to diet or as a result of IM metabolism, leads to a decrease in VLDL formation and triglyceride export from the liver, resulting in the development of a fatty liver. Choline is also an essential component of the mitochondrial membrane.[44] Choline deficiency decreases the mitochondrial membrane concentrations of phosphatidylethanolamine and phosphatidylcholine, resulting in decreased membrane potential, which, in turn, causes oxidative damage.[44]

The IM may contribute to decreased choline bioavailability[46] by metabolising dietary choline found in eggs, milk and red meat into trimethylamine (TMA).[47] This increases the production of TMA, which is absorbed into the blood, and has been associated with an increased risk of cardiovascular disease.[47] In addition, once TMA reaches the liver it is further metabolised by flavin-containing monooxygenases 1 and 3 to generate trimethylamine-N-oxide (TMAO).[47–49] This may lead to increased hepatic triglyceride accumulation as TMAO effects BA pool size by decreasing BA synthesis through the inhibition of key enzymes and by limiting the enterohepatic circulation of BAs through repression of the organic anion transporter and multidrug resistance family protein expression.[50–52] Therefore, it is possible that choline deficiency, either through the diet or the conversion of IM to TMA, may cause fat to accumulate in the liver.

Ethanol

Ethanol, which comes primarily from food and beverages, is absorbed through the mucosa of the gastrointestinal tract.[53] However, ethanol can also be produced and metabolised by the IM in the absence of alcohol consumption.[54] Ethanol is formed from *Escherichia coli* and under anaerobic conditions during the fermentation of carbohydrates, *E. coli* can metabolise pyruvic acid to generate acetaldehyde, which can be reduced to ethanol.[55] Acetaldehyde has been shown to decrease the gut barrier function by weakening tight junctions and therefore facilitates the translocation of microbial products into the systemic circulation.[56,57] Furthermore, studies have shown that acetaldehyde can stimulate an inflammatory and adaptive immune response by downregulating antimicrobial peptide expression in the intestine,[58–61] thus leading to further hepatic injury.

Taken together, the IM and bacterial products can directly and indirectly affect the liver through various mechanisms (Fig. 1), leading to a wide variety of liver diseases (Fig. 2).

Gut bacteria and specific liver diseases

Non-alcoholic fatty liver disease

NAFLD is one of the most common causes of liver disease worldwide, affecting 15–30% of the general population.[62–65] NAFLD ranges from simple fat deposition in the liver (steatosis) to inflammation (non-alcoholic steatohepatitis or NASH) to fibrosis and cirrhosis.[66] Research studies have shown that altered IM composition, so-called “dysbiosis”, contributes to the pathogenesis of NAFLD,[52,67–69] however causality has yet to be proven.

Despite a large number of preclinical data investigating and demonstrating a relationship between dysbiosis and NAFLD, only a limited number of human studies, mostly cross-sectional, have investigated the role of the IM in NAFLD, with variable results. In adults, patients with NASH were found to have lower amounts of Bacteroidetes, independent of body mass index and diet.[67] Studies have shown that there are differences in the IM between patients with NAFLD and healthy controls.[68–71] One study found that NAFLD severity is associated with IM dysbiosis and shifts in the metabolic function of the IM.[72] Specifically, they found that the abundance of *Bacteroides* was independently associated with NASH and *Ruminococcus* with fibrosis.[72] More recently, another cross-sectional study found that those with NAFLD had significantly decreased Bacteroidetes and Firmicutes, along with increased *Lactobacillus* compared with healthy controls, while those with NASH had decreased *Ruminococcus,*

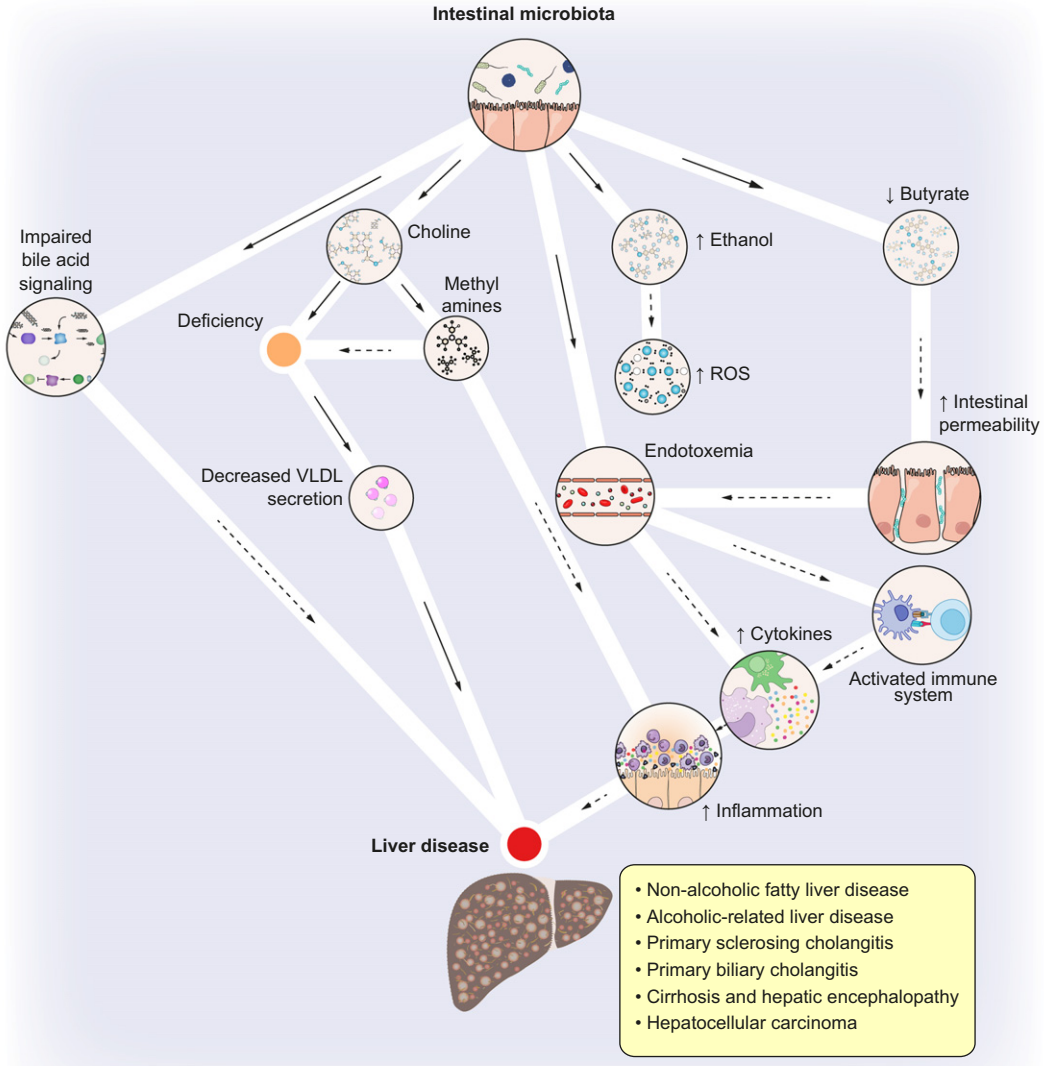

Fig. 1. Mechanisms in which the intestinal microbiota can affect the liver. ROS, reactive oxygen species; VLDL, very-low density lipoprotein.

Faecalibacterium prausnitzii and *Coprococcus* compared to healthy controls, independently of body mass index and insulin resistance.[73] In paediatrics, results showed an increased abundance of *E. coli* in patients with NASH compared to healthy controls, which was associated with higher blood alcohol levels.[69] One intervention study, which included 15 adult women placed on a choline-deficient diet found that pre-diet microbiota composition, specifically a lower abundance of *Gammaproteobacteria* or a higher abundance of *Erysipelotrichi* increased vulnerability to the development of a fatty liver during choline depletion.[52] Furthermore, they found that host genotypes (single nucleotide polymorphism in the *PEMT* gene) and specific IM can predict choline deficiency-induced fatty liver (assessed by magnetic resonance imaging).[52] One study assessed faecal ester volatile organic compounds and found that specific patterns were associated with differences in the IM when patients with NAFLD, diagnosed on ultrasound, were compared to controls.[68] Recently, a study investigating the relationship between the IM and immune function in NAFLD found that specific immune cells in the portal or lobular areas correlated with specific faecal IM. Specifically, *Faecalibacterium prausnitzii* was negatively correlated with $CD45^+$ and $CD163^+$ cells in the portal tract and *Prevotella* was negatively correlated with $CD20^+$ cells in the liver lobule.[74] Taken together, several studies showed associations between the IM or bacterial products and NAFLD.

Alcohol-related liver disease

ALD occurs in patients who chronically abuse alcohol. Like NAFLD, non-progressive ALD is characterised by fat accumulation in the liver, whereas progressive ALD (alcoholic steatohepatitis) exhibits

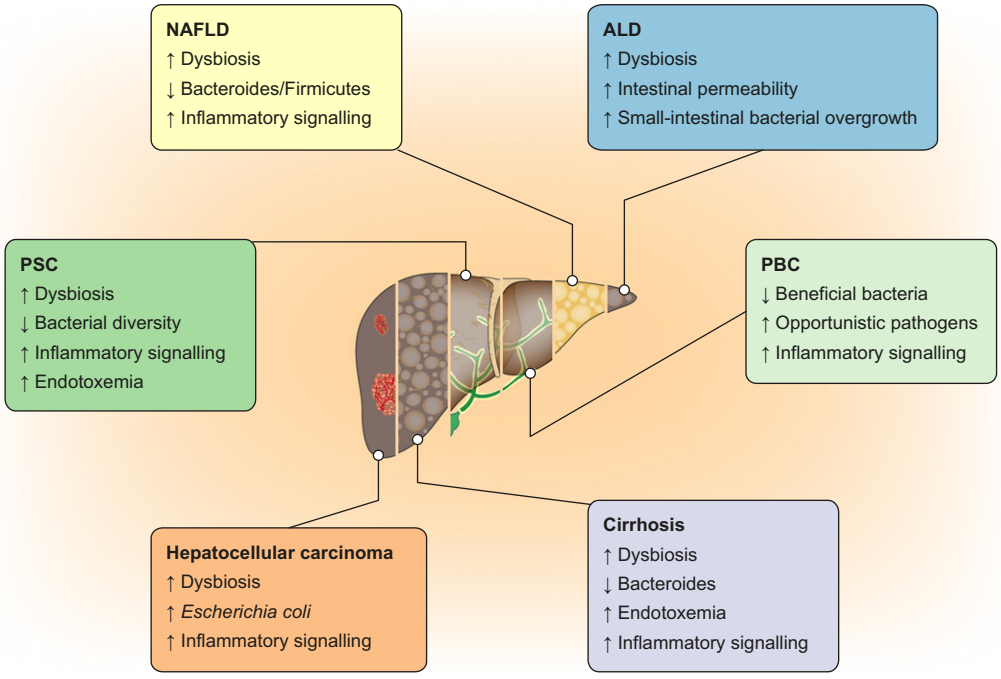

Fig. 2. The role of intestinal microbiota in liver disease. ALD, alcohol-related liver disease; NAFLD, non-alcoholic fatty liver disease; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis.

hepatic inflammation.[75] Recently, research has investigated the role of the IM in ALD, specifically focussing on how alcohol can cause microbiota-related dysbiosis which in turn, may contribute to the pathogenesis of ALD.

Many studies at the preclinical and experimental level have shed light on the relationship between ALD and dysbiosis. Through these many studies, multiple pathogens, toxic components and pathways have been shown to participate in the development of ALD. The amount of clinical data is unfortunately not as extensive.

Studies including both mouse models and human participants found that alcohol consumption provokes a change in the IM leading to dysbiosis.[76] Specifically, patients with ALD have lower levels of *Bifidobacterium*, *Enterobacterium* and *Lactobacillus* spp.,[77–81] while cirrhotic patients with ALD exhibit a significant reduction in Bacteroidetes and Firmicutes phyla.[78,82–84] On the other hand, the Proteobacteria, Fusobacteria and Actinobacteria phyla were increased.[82] Other studies using faecal samples from alcoholic patients showed a reduction in the *Lactobacillus* spp.,[85] whereas cirrhotic patients were shown to have lower faecal amounts of *Bifidobacterium* spp..[85–87] When comparing IM of alcoholics with liver cirrhosis to alcoholics without cirrhosis, it was found that the IM of those with cirrhosis contained more *Enterobactericeae.*[78,79] Based on some of these findings, the term cirrhosis dysbiosis ratio (CDR) was suggested,[88] representing the ratio of autochthonous or beneficial bacteria to potentially pathogenic bacteria,[89] with a low ratio correlating with a more advanced disease state. Compared to other aetiologies of cirrhosis, ALD had the lowest ratio.[88]

Studies have also demonstrated an increase in the overall number of organisms in the small bowel of alcoholic patients.[90–92] An evaluation of chronic alcoholics compared to patients without a history of alcohol abuse, using the hydrogen breath test, showed a significantly higher prevalence of small-intestinal bacteria overgrowth (SIBO) in alcoholics compared to controls. However, no differences were found between alcoholic patients with liver cirrhosis and those without liver cirrhosis.[90] The presence of SIBO has been shown to significantly correlate with a higher prevalence of spontaneous bacterial peritonitis and with the severity of alcoholic cirrhosis.[92] These changes in the IM of alcoholic patients seem to be accompanied by changes in colonic pH and liver steatosis.[77] It also correlates with a higher level of serum endotoxin and increased intestinal TNF-α levels, as well as increased levels of nitric oxide, IL-6, and IL-8.[93–95] Other studies also found higher levels of bacterial products in the blood of alcoholic patients compared to healthy controls.[88,96] Additionally, endotoxemia after acute alcohol intoxication has been shown to correlate with haemodynamic derangement in cirrhotic portal hypertension.[94,97,98] These findings suggest a potential link between dysbiosis and ALD, with alcohol promoting dysbiosis and leading to increased gut barrier permeability, consequently causing translocation of IM and endotoxins into the portal circulation, and eventually the liver. This, in turn, triggers hepatic inflammation and liver damage, particularly through the

interaction between lipopolysaccharides (LPS) and TLRs.[99]

Primary sclerosing cholangitis

Primary sclerosing cholangitis (PSC) is characterised by inflammation and scarring of the bile ducts.[100] The few studies investigating the IM in PSC have shown an overall reduction in IM diversity, however, there are inconsistencies in these findings at the genus and species level[100]

The evaluation of the IM in patients with PSC and PSC-inflammatory bowel disease (IBD) demonstrated low bacterial diversity,[101] and an overrepresentation of *Rothia*, *Enterococcus, Streptococcus*, *Clostridium*, *Veillonella* , *Haemophilus*, *Fusobacterium* and *Lactobacillus* genera regardless of concomitant IBD.[101,102] Another study confirmed that *Veillonella* abundance was markedly increased in PSC compared to healthy controls.[103] Studies looking at intestinal biopsies found that the overall microbiota profile of those with PSC was characterised by enrichment of *Barnesiellaceae* and a reduction in *Clostridiales.*[104,105]

According to the aforementioned studies, these changes lead to IM dysbiosis and are associated with the pathogenesis of PSC by inducing bacterobilia, which in turn activates a proinflammatory pathway in the cholangiocytes leading to fibrosis and inflammation. Bacterobilia may also play a role in molecular mimicry, through endotoxemia, leading to the creation of antibodies and causing immune-mediated biliary damage.[106,107]

Primary biliary cholangitis

Primary biliary cholangitis (PBC) is a disease that results in the progressive destruction of the bile ducts within the liver.[108] In a cross-sectional study comparing urodeoxycholic acid (UDCA) treatment-naïve patients with PBC and healthy controls, dysbiosis was observed in PBC and was partially reversed by UDCA. Bacteroidetes spp. were significantly decreased and Fusobacteria, *Haemophilus, Veillonella, Clostridium, Lactobacillus, Streptococcus, Pseudomonas, Klebsiella,* an unknown genus in the family of Enterobacteriaceae and Proteobacteria spp. were over-represented in comparison to healthy controls.[108] Another study comparing patients with PBC to healthy controls found that patients with PBC had depleted levels of potentially beneficial gut bacteria, including Acidobacteria, *Lachnobacterium* spp., *Bacteroides eggerthii* and *Ruminococcus bromii*, but higher levels of bacterial taxa containing opportunistic pathogens, such as y-Proteobacteria, *Enterobacteriaceae*, *Neisseriaceae*, *Spirochaetaceae*, *Veillonella*, *Streptococcus*, *Klebsiella*, *Actinobacillus pleuropneumoniae*, *Anaeroglobus geminatus*, *Enterobacter asburiae*, *Haemophilus parainfluenzae*, *Megasphaera micronuciformis* and *Paraprevotella clara.*[109] They also found that this PBC-related alteration in the IM was associated with increased liver injury indicators and serum inflammatory cytokines, thus suggesting that the altered IM may be involved in the onset or development of PBC.[109]

The potential IM-related mechanisms behind the progression of liver disease are similar to the aforementioned mechanisms previously described for PSC.[107]

Cirrhosis and hepatic encephalopathy

Cirrhosis is considered end-stage liver disease that is characterised by severe fibrosis and a loss of liver cells. Each of the aforementioned liver diseases can result in a cirrhotic liver.[110] Research has found that patients with cirrhosis have lower levels of Bacteroidetes and higher levels of Proteobacteria, *Enterococcus, Veillonella, Megasphaera, Burkholderia, Prevotella and Fusobacteria.*[81,111] In addition, cirrhotic patients also have lower levels of autochthonous taxa such as *Blautia, Roseburia, Faecalibacterium, Dorea,* Lachnospiraceae and *Ruminococcaceae*.[81,111] When analysing the duodenal mucosal microbiota of 30 cirrhotic patients, Chen *et al.* found that cirrhotic patients' colonisation was significantly different than that of 28 healthy controls.[111] There seemed to be an overrepresentation of *Veillonella*, *Megasphaera*, *Dialister*, *Atopobium*, and Prevotella in cirrhotic patients compared to controls. *Veillonella*, *Prevotella, Neisseria,* and *Haemophilus*, were the taxa best able to discriminate between those with cirrhosis and healthy controls. Other studies have demonstrated higher levels of buccal-derived microbiota in the stool samples of patients with cirrhosis, as well as a significantly altered salivary microbiome in cirrhotic patients.[112,113] This could suggest that the oral microbiota has a great impact upon duodenal and possibly intestinal microbiota in this population. Another author even mentioned the possibility that the duodenal microbiota might directly contribute to hepatic encephalopathy in cirrhosis.[111]

Hepatic encephalopathy, which is defined as cognitive impairment, occurs as a result of severe liver disease. Studies have found that there is a link between hepatic encephalopathy and by-products of the IM, specifically endotoxemia.[114] One study compared the IM in patients with hepatic encephalopathy to other cirrhotic patients and healthy controls and found that those with hepatic encephalopathy had higher levels of Alcaligenaceae, Enterobacteriaceae and Fusobacteriaceae along with lower Ruminococcaceae and Lachnospiraceae.[114] Of those, Alcaligenaceae and Porphyromonadaceae were positively correlated with cognitive impairment whereas *Prevotella* was linked to improvement of cognition and decreased inflammation.[114] Their study also showed a higher concentration of Veillonellaceae, endotoxemia and inflammation in patients with hepatic encephalopathy.[114] Another study demonstrated that the composition of the IM could predict decompensation and hospitalisation of cirrhotic patients.[88]

Higher serum endotoxin levels, lower CDR and increased pathogenic taxa were significantly linked to death secondary to multi-organ failure when compared to patients who survived. In the same study the salivary microbiome was shown to independently correlate with liver-related 90-day hospitalisation regardless of the model for end-stage liver disease (MELD) score or the status of hepatic encephalopathy.[88]

Several mechanisms have been suggested for the association between IM and cirrhosis that include increased small bowel permeability and decreased small bowel motility, leading to small bowel overgrowth. This in turn leads to translocation of the IM and endotoxins into the portal circulation, activation of inflammatory pathways in HSCs through TLR4 and subsequently the development of fibrosis.[115] For HE, ammonia plays a central role in the development of the disease. Some studies have shown that in patients with cirrhosis, in addition to bacterial translocation and activation of proinflammatory cytokines, there is an increased quantity of urease active bacteria, which would lead to increased production of ammonia in the small bowel and increased levels in the portal blood.[115,116]

Hepatocellular carcinoma

HCC can be a complication of many liver diseases. Dysbiosis may contribute to HCC pathogenesis by increasing steatosis, oxidative stress and inflammation.[14]

Changes in the microbiota have been suspected of playing a role in carcinogenesis. One study by Grat *et al.* investigated the IM of 15 patients with HCC undergoing liver transplantation and compared them to 15 patients who did not have HCC but had a similar aetiology of cirrhosis and a similar MELD stage. The study showed that the presence of HCC was significantly associated with increased faecal *Escherichia coli*.[117] Another study evaluated liver tissue samples in patients with HCC and found the presence of *Helicobacter* spp., suggesting intestinal translocation as a potential mechanism for carcinogenesis.[118] However, *Helicobacter* could not be found in patients with viral-induced HCC.[118]

Mechanistically, murine studies suggested that the IM can contribute to HCC pathogenesis through its interaction with the TLRs, particularly TLR4. However, more clinical research is needed to further characterise the causal role of the IM in the pathogenesis of HCC.[119]

Limitations to IM and liver disease studies

Research in the area of IM and liver diseases is rapidly evolving, but there are several limitations to consider when interpreting this association. First of all, differences in genetics[120] and environmental factors such as diet,[121] alcohol[86] and medication/antibiotic use[122] have been shown to contribute to variations in IM. Additionally, the use of different liver diagnostic tools that are used for primary endpoints in clinical trials is another limitation. Some studies will use a liver biopsy,[67] the gold standard for diagnostics, while others use non-invasive and less reliable tools such as imaging or blood tests,[68] which could explain the differences seen in clinical research. Another limitation in human IM studies is how the stool sample is collected. Although similar IM phyla predominate across the stomach, small intestine and colon, there are variations in IM composition and abundance.[123] The majority of human studies analyse stool samples, however 1 study found differences in the IM when comparing stool samples to caecal luminal contents,[124] therefore limiting the generalisability of the results. Furthermore, there are variations in the sequencing methods used, which all produce different results. These include quantitative PCR,[67] 16S rRNA sequencing[72] or shotgun sequencing.[112] Additionally, differences in bioinformatic analysis platforms, such as QIIME,[125] Mothur[126] and PICRUST,[127] can contribute to variations in results. Overall, it is important to consider these limitations when analysing IM and liver disease research and they should be considered when designing future studies.

Future directions

Based on the above studies, there is likely an association between the IM and liver disease, but a causal relationship has yet to be confirmed. Several studies looking at the effect of IM manipulation by pre-, pro- and synbiotics, as well as faecal microbiota transplant (FMT), suggest that the IM has a role in liver diseases.

Pre- and probiotic treatment

Several studies have investigated the role of pre- and probiotic treatment in patients with liver disease. A summary of these studies can be found in Table 1. However, no studies have been carried out in patients with PSC or PBC.

Overall, pre-, pro- and synbiotics seem to improve various liver parameters in patients with NAFLD, ALD, cirrhosis or HE, supporting a role for the IM in liver disease pathology. However, interventions vary in terms of the product type and amount used and most of the studies have small sample sizes. Therefore, more research is needed with larger randomised controlled trials before any recommendations can be made. Answers may come from the clinical trials currently being conducted; for studies currently recruiting see Table 2.

Faecal microbiota transplantation

FMT has recently become a standard of care for treating antibiotic-resistant *Clostridium difficile*.[144,145] As a result, FMT is becoming frequently investigated as a potential therapeutic option for a variety of diseases, including those that are liver related. As previously stated, the liver-gut axis

Table 1. Summary of pre-, pro- and synbiotic liver disease studies.

Disease	Treatment	Study design	Outcome	Reference
NAFLD	VSL#3 (combination of 8 probiotic strains) or placebo for 4-months	RCT n = 48 children	VSL#3 improved NAFLD	128
NAFLD	Multi-probiotic product or placebo	RCT n = 58 adults	Probiotic resulted in reductions of AST, GGT, TNF-a and IL-6	129
NAFLD	Synbiotic or placebo	RCT n = 50	Synbiotic group had significant decrease in AST, total cholesterol, triacylglycerol and steatosis (based on Fibroscan)	130
ALD	*Bifidobacterium bifidum and Pacobacillus* plantarum daily for 5 days	Open-label randomised n = 66	Reductions in AST and ALT	85
ALD	*Bifidobacterium bifidum, Bifidobacterium lactis, Bifidobacterium ongum, Lactobacillus acidophilus, Lactobacillus rhamnosus* GG, *Streptococcus thermophiles* $5x10^9$ 1 capsule twice daily for 28 days or placebo	Double-blind RCT n = 50	Decrease in SIBO, however no difference in intestinal permeability	131
ALD/HE	VSL#3 or placebo	RCTl n = 130	No difference in incidence of encephalopathy or mortality. However, reductions in Child-Pugh, MELD, plasma TNF-a, IL-1B and IL-6 seen	132
ALD/Cirrhosis	*Lactobacillus subtilis* and *Streptococcus faecium* (daily for 7 days) or placebo	Double-blind RCT n = 117	Decrease in TNF-a and an increase in albumin levels. Stabilisation of LPS levels in cirrhotic patients	133
ALD/Cirrhosis	*Lactobacillus casei* Shirota ($6.5x10^9$) three times a day for 4 weeks	Open-label n = 12	Normalised neutrophil phagocytic capacity. No improvement in disease control and no change on TNF-a and IL10	134
ALD/Cirrhosis	VSL#3 1 sachet daily for 60 days or placebo	Double-blind RCT n = 63	Reduction of hepatovenous pressure gradient and TNF-a	135
ALD/Cirrhosis	VSL#3 2 sachets twice a day for 60 days	Open pilot study n = 8	Trending reduction of endotoxin levels and TNF-a	136
ALD/Cirrhosis	VSL #3 2 sachets twice daily for 60 days or placebo	Double-blind RCT n = 11 ALD and n = 15 cirrhosis	No impact on IM, endotoxins, liver function, hepatovenous pressure. Reduction in plasma aldosterone.	137
Cirrhosis/HE	*Lactobacillus rhamnosus* GG twice daily for 8 weeks or placebo	RCT n = 30	No change in cognition. However, decrease in endotoxemia and TNF-a.	138
Cirrhosis/HE	VSL#3 1 capsule three times a day for 3 months or placebo	RCT n = 86	Reduction of ammonia, SIBO and OCTT. Increased psychometric HE scores and CFF threshold. Significantly less patients developed overt HE.	139
Cirrhosis	*Escherichia coli Nissle* for 42 days	Double-blind RCT n = 39	Improvement in intestinal colonisation. Lowering of endotoxemia and Improvement of liver function/Child-Pugh score.	140
Cirrhosis/HE	Lactulose and lactitol	Cochrane review of randomised control trials n = 1828	Beneficial effect of non-absorbable disaccharides on mortality, HE, reduction of serious adverse events associated with liver disease (liver failure, hepatorenal syndrome, variceal bleed)	141
Cirrhosis/HE	*Bifidobacterium longum* plus fructo-oligosaccharides for 90 days or placebo	Double-blind RCT n = 60	Decrease in ammonium (NH_4) and performance on Trial Making Test A and B. Significant improvement of symbol digit modalities test, block design and MMSE	142
Cirrhosis/HE	Synbiotic treatment daily for 30 days or placebo	RCT n = 55	Increase in faecal Lactobacillus. Reduction in endotoxemia, blood ammonia and reversal of minimal HE in 50%. Improvement of Child-Pugh class in 50%.	143

ALD, alcohol-related liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CFF, critical flicker frequency; GGT, gamma-glutamyl transferase; HE, hepatic encephalopathy; IL-, interleukin-; IM, intestinal microbiota; LPS, lipopolysaccharide; MELD, model for end-stage liver disease; MMSE, mini-mental state examination; NAFLD, non-alcoholic fatty liver disease; OCTT, orocecal transit time; RCT, randomised controlled trial; SIBO, small intestine bacteria overgrowth; TNF-a, tumour necrosis factor-alpha.

plays an essential role in the pathogenesis of liver disease, with recent research suggesting that FMT could be beneficial. A pilot study, investigating the effects of FMT in 8 male patients with severe alcoholic hepatitis compared to historical controls found that there were marked improvements in liver disease within 1 week. This included the resolution of ascites and hepatic encephalopathy in the majority of patients. They also saw significantly improved 1-year survival rates compared to matched controls receiving standard of care (87.5% *vs.* 33.3%).[146] A recent clinical trial investigating whether FMT improves hepatic encephalopathy compared to standard of care in male patients with cirrhosis and recurrent hepatic encephalopathy, found that those receiving FMT had reduced hospitalisation rates and improved cognition and dysbiosis. Furthermore, in the 5 months

Table 2. Ongoing pre-, pro- and synbiotic trials.

Type of liver disease	Type of pre-, pro or synbiotic	Study design	Primary outcome	Location	ClinicalTrial.gov ID
NAFLD	2x probiotic/day: *Lactobacillus acidophilus* $1x10^9$ CFU + *Bifidobacterium lactis* $1x10^9$ CFU + *Lactobacillus rhamnosus* $1x10^9$ CFU + *Lactobacillus paracasei* $1x10^9$ CFU	RCT; n = 46 adults	Change in fibrosis by hepatic elastography	Brazil	NCT03467282
NAFLD	1x probiotic/day: *Lactobacillus acidophilus* 10^9, B. lactis 10^9	RCT; n = 58 adults	Hepatic changes based on FIBROMAX test	Brazil	NCT02764047
NAFLD	Synbiotic: Fructo-oligosaccharide with a degree of polymerisation <10 at 4 g/twice a day plus *Bifidobacterium animalis* subsp. lactis BB-12 as minimum of 10 billion CFU/day (1 capsule a day).	RCT; n = 100 adults	Change in liver fat by MRS	United Kingdom	NCT01680640
NAFLD	2x prebiotic/day: oligofructose-enriched inulin (Synergy1)	RCT; n = 60 adults	Change in liver fat by MRI, change in liver fibrosis by FibroScan, change in liver injury by Fibrotest Score	Canada	NCT02568605
NAFLD	Prebiotic 16 g/day: inulin and oligofructose	RCT; n = 60 adults	Change in liver fat by MRS, biochemistry	Israel	NCT02642172
Acute alcoholic hepatitis	1x probiotic/day: *Lactobacillus rhamnosus* GG	RCT; n = 130 adults	MELD Score	United States of America	NCT01922895

CFU, colony forming units; MRS, magnetic resonance spectroscopy; MRI, magnetic resonance imaging; NAFLD, non-alcoholic fatty liver disease; RCT, randomised controlled trial.

following the FMT procedure, no FMT recipients' developed hepatic encephalopathy, whereas 5 of those receiving standard of care did.[147] Relevant to NAFLD, 1 pilot study reported that FMT significantly reduced insulin resistance associated with changes in the IM.[148] Again, more clinical trials are needed

Table 3. Current faecal microbiota transplantation trials.

Type of liver disease	Type of faecal microbiota transplantation	Study design	Primary outcome	Location
NASH	Frozen faecal material from lean healthy donors infused into the duodenum by endoscopy	Open label; n = 5 adults	Change in liver fat by MRI	United States of America
NASH-related cirrhosis	Recipient will receive healthy donor faecal samples through a naso-gastric tube, 100 ml once a month for 5 months.	RCT; n = 60 adults	Reduction in hepatic venous pressure gradient	India
Alcoholic hepatitis	Healthy donor FMT administered by naso-gastric tube for 7 days	RCT; n = 130 adults	Proportion of participants with Overall Survival at 3 months	India
Cirrhosis	FMT by endoscope and/or enema	RCT; n = 60 adults	Number of adverse events complication rate	China
Cirrhosis	FMT (200 ml) from donated healthy samples will be administered into the duodenum via a gastroscope	RCT; n = 32 adults	Assessment of the feasibility of FMT and assessment of the incidence of treatment-emergent adverse events	United Kingdom
Cirrhosis	One-dose of 90 ml of FMT enema from healthy donor stool sample	RCT; n = 20 adults	Proportion of participants with a related serious adverse event, with newly acquired transmissible infectious diseases and related adverse event	United States of America
Hepatic encephalopathy	Single-arm open-label healthy donor FMT administered at Week 0 by colonoscopy and at Weeks 1-4 by enema	Open label; n = 10 adults	Time to hepatic encephalopathy breakthrough	Canada
Hepatic encephalopathy	Single-centre open-label trial of RBX2660 (microbiota suspension). Healthy donor FMT administered at Week 0 by colonoscopy and at Weeks 1-4 by enema	Open label; n = 30 adults	Time to hepatic encephalopathy breakthrough	Canada
Hepatic encephalopathy	Subjects will receive 15 oral capsules of FMT on days 1, 2, 7, 14, and 21. FMT prepared from healthy donors.	RCT; n = 30 adults	Psychometric Hepatic Encephalopathy Score	United States of America
Acute liver failure	FMT administered by enema	RCT; n = 40 adults	Survival	India

FMT, faecal microbiota transplantation; MRI, magnetic resonance imaging; NAFLD, non-alcoholic fatty liver disease; RCT, randomised controlled trial.

to fully investigate the beneficial effects of FMT on specific liver diseases, several of which are underway (Table 3).

Conclusion

There is evidence of associations between dysbiosis and liver disease, particularly as it relates to NAFLD, ALD, cirrhosis and hepatic encephalopathy. Specifically, molecules produced by the IM such as endotoxin and proinflammatory cytokines play a role in the pathogenesis of liver diseases. Furthermore, the IM can be influenced by several environmental factors, particularly diet and alcohol in the case of NAFLD and ALD Other than dietary changes or alcohol abstinence, manipulations of the IM by various interventions show promise. The majority of studies investigate the use of pre-, pro- and synbiotics in NAFLD, ALD and cirrhosis/HE and have found that these products improved clinical and biochemical markers of liver disease, however studies in patients with PSC and PBC are lacking. In conclusion, even though these studies show promise, more clinical research is required, particularly larger randomised controlled trials to bridge the gap between experimental/preclinical data and the small amount of human data on the subject.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at https: https://doi.org/10.1016/j.jhepr.2019.04.004.

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